Using HHsearch to tackle proteins of unknown function – a pilot study with PH domains

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Supplemental Information

Supplemental Table 1.

Families of PH-like domains classified by different profile--sequence tools

Family Name	grouping in other databases			# entries in PDB		Sample structure
	CDD	SCOP	Pfam family	groups	full	
classical PH	classical PH (cPH)	PH	PH, PH2-13, IQ_Sec7 Mcp5[Num1]	115	1=96 2=59 3=26	2coa_A 1txd_A(C) 3ohm_A
FERM-C	FERM-C	FERM (3rd domain)	FERM_C	14	1=10 2=4	3pvl_A 2j0j_A
PTB	PTB, PID, IRS	PTB	PTB, PID, PID2, ICAP1integrinBP	23	1=22 2=21	4wj7_A 2cy5_A
RBD	Ran-BP1/BD WH1 YRB1	RanBD, Enabled/VASP hom.	WH1, Ran-BP1	26	1=23 2=22	2qkl_A 1ddw_A
—	Dcp1	DCP1	DCP1	3		1q67_A
AVO1 (Torc2 subunit)	PH_Avo1	X	SIN1	2		3voq_A
Bact-PH	bPH	BPHL	bPH1-6	2		3hsa_A
CARM1-PH	CARM1	X	CARM1	2		2oqb_A
CARMIL	X	X	CARM1	1		4k17_A
DOK-PTB	PH_DOK1,2,3	PTB	PTB, IRS	7		1j0w_A
GLUE (Vps36)	Vps36	VPS36 N-term-like	Vps36_ESCRT-II	3		2cay_A
GRAM	GRAM	GRAM	GRAM, BBL5(DM16)	4		1lw3_A
hSac2-PH	X	X	hSac2	1		4xuu_A
ICln	Icln	X	Voldacs	3		1zyi_A
ISP3	X	X	X	2		4chj_A
Myosin1c-TH1	Myosin_TH1 (PH-like)	X	Myosin_TH1	1		4r8g_A
Necap	Necap1	Necap1 N-term	DUF1681	1		1tqz_A
NF1-PH	PH-neurofibromin1 =NF1	X	X	2		3pg7_A
OCRL1 & Inpp5b PH	PH_OCRL-like	X	X	2		2kig_A & 2kie_A
POB3N-PH	X	X	SSrecog	2		4khb_D(N)
Pre-BEACH	PH_BEACH	Pre-BEACH	PH_BEACH	2		1t77_A
RGS-RhoGEF-PH	X	X	X	1		4gou_A
Rpn13	Rpn13	X	Proteasome Rpn13	3		2r2y_A
RTT106C	Rtt106	X	Rtt106	3		3fss_A (C)
SEC3-PH	PH-Sec3_like	X	Sec3-PIP2_bind	2		3hie_A
Sharpin-PH	PH_Sharpin	X	Sharpin_PH	1		4emo_A
SNX17(FERM-C)	FERM-likeC_SNX17	X	X	1		4gxb_A
split (a-syntrophin)	PHsplit_syntrophin	a1syntrophin	PH	3		2adz_A
SPT16D	FACT(SPT16/CDC68)	X	SSrecog	1		4khb_A
Spt16M	PH2_SSRP1-like rpt1	SSRP1-like	SSrecog	3		4kho_A (N)
SSrecog	PH2_SSRP1-like rpt2	FACT Pob3M	SSrecog	1		3fss_A(C)
TFIIH	TFIIH	TFIIH	PH_TFIIH	2		1pfj_A
USP37-PH	PH_USP37_like	X	UCH_N	1		3u12_A
ZF21-PH	X	X	ZFYVE21_C	1		2rrf_A
Subfamilies:	**>350**	**15**	**39 + 1 wrong**	**34**	**39**	
WRONG (*i.e.* not PH-like)			DUF1126 (EF hand)			

Supplemental Table 1. Classifications of PH-like domains.
Information on our classification of 39 PH-like families (see Figure 2), starting with the major groups that contain overlapping families in some classifications. Our classification is compared to those in Conserved Domain Database (CDD) at NCBI, the Structural Classification of Protein (SCOP) database at scop.berkeley.edu/, and Pfam 30.0. RED indicates PH-like families that were missing from the PH-like clan. "X" indicates that the family was missing completely. The penultimate column shows the number of PH-like domains for each family in PDB (using a version non-redundant at 70% identity), both for grouped families (excluding extensive overlap, n=34) or the full list (n=39). The final column shows the one structure we chose to represent the family for searches in PSI-BLAST and HHsearch. Where the structure includes more than one PH-like domain, the segment used is indicated. The bottom lines show the total number of PH-like families in each classification, and information on one domain wrongly assigned as PH-like in Pfam.

Supplemental Table 2: details of 91 yeast PH-like domains

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A Known PH-like proteins	systematic gene name	protein length	residues	method 1	2	3	4		5	note
				InterPro -Scan	SMART	sup-fam	PDB-to-yeast p[SS]	PDB-to-yeast family	yeast -to-PDB	
Ask10p	Ygr097	1146	477-725	cPH	cPH	cPH	92.4	cPH[1/3]	99.9	h
Atg26p-1	Ylr189c	1198	165-340	cPH	cPH	cPH	99.2	cPH[1/2/3]	99.9	h
Atg26p-2	Ylr189c	1198	575-682	gram	gram	–	98.1	gram	97.6	h
Avo1p	Yol078w	1176	1065-1172	–	+	–	100.	PTB[Avo1]	100.0	c
Bem2p	Yer155c	2167	1780-1957	cPH	cPH	cPH	92.7	cPH[1/2/3]	99.8	h
Bem3p	Ypl115c	1128	630-745	cPH	cPH	cPH	99.1	cPH[split]	99.9	h
Boi1p	Ybl085w	980	778-895	cPH	cPH	cPH	99.5	cPH[1/2/3]	99.9	h
Boi2p	Yer114c	1040	769-889	cPH	cPH	cPH	99.5	cPH[1/2/3]	99.9	h
Bph1p	Ycr032	2167	1370-1467	BPH	BPH	BPH	99.8	pre-	93.4	c
Bud4p	Yjr092w	1448	1300-1420	cPH	cPH	cPH	98.9	cPH[1/2/3]	99.9	h
Caf120p-2 °	Ynl278w	1060	74-214	cPH	cPH	cPH	93.1	cPH[1/3]	99.9	h
Cdc24p	Yal041w	854	484-682	cPH	cPH	cPH	98.1	cPH[1/2/3]	99.7	h
Cla4p	Ynl298w	842	60-183	cPH	cPH	cPH	98.9	cPH[1/2/3]	99.9	h
Dcp1p	Yol149w	231	42-223	+	Dcp1	Dcp1	100.	Dcp1	100.0	c
Exo84p	Ybr102c	753	352-443	cPH	cPH	cPH	98.6	cPH[1/2]	99.8	c
Far1p	Yjl157c	830	444-540	–	–	–	95.2	cPH[2]	**79.1**	h
Ira1p	Ybr140c	3092	2204-2302	–	–	–	99.9	cPH[NF1]	100.0	h
Ira2p	Yol081w	3079	2205-2301	–	–	–	99.9	cPH[NF1]	100.0	h
Lam4p	Yhr080c	1345	535-661	gram	gram	–	97.3	gram	96.6	h
Lam5p	Yfl042c	674	223-305	gram	gram	–	97.9	gram	95.9	h
Lam6p	Ylr072w	693	170-270	gram	gram	–	97.7	gram	96.4	h
Las17p	Yor181	633	21-134	RBD	RBD	RBD	99.5	RBD[2]	100.0	c
Lmo1p	Yll007c	665	462-600	–	–	–	97.1	cPH[3]	100.0	h,o
Lot5p	Ykl183w	171	50-190	ICln	ICln	–	99.9	pICln	99.8	h
Mdr1p	Ygr100	950	40-157	gram	gram	gram	97.5	gram	98.0	h
Myo3p	Ykl129c	1272	807-911	–	–	–	99.7	cPH[4r8g]	96.3	h
Myo5p	Ymr109	1219	808-911	–	–	–	99.8	cPH[4r8g]	97.2	h
Num1p	Ydr150	2748	2572-2687	cPH	cPH	cPH	98.1	cPH[1/3]	99.9	h
Nup2p	Ylr335w	720	602-718	RBD	RBD	RBD	99.9	RBD[2]	100.0	h
Nvj2p	Ypr091c	770	125-268	cPH	cPH	cPH	#N/A	cPH[1/2]	99.9	h
Opy1p-1	Ybr129c	328	65-155	cPH	cPH	cPH	99.8	cPH[1/2/3]	99.9	h
Opy1p-2	Ybr129c	328	213-320	cPH	cPH	cPH	99.8	cPH[1/2/3]	99.9	h
Osh1p	Yar042	1188	280-383	cPH	cPH	cPH	99.1	cPH[1/2/3]	99.9	h
Osh2p	Ydl019c	1283	288-390	cPH	cPH	cPH	99.3	cPH[1/2/3]	99.9	h
Osh3p	Yhr073	996	220-318	cPH	cPH	cPH	99.5	cPH[1/2/3]	99.9	h
Plc1p	Ypl268w	869	122-217	+	812	cPH	99.4	cPH[1/2/3]	99.9	h,o
Pob3p-1	Yml069	552	01-111	+	–	–	94.2	Pob3N	100.0	c
Pob3p-2	Yml069	552	102-232	+	–	–	100.	Pob3N	100.0	c
Pob3p-3	Yml069	552	247-347	+	+	+	96.9	SSrecog	100.0	c
Pob3p-4	Yml069	552	365-470	Rtt106	Rtt106	Rtt106	99.7	Rtt106C	100.0	c
Psy2p	Ynl201c	858	28-127	+	PH	RBD	99.5	RBD[2]	100.0	h
Rgc1p	Ypr115	1083	493-587	cPH	cPH	cPH	89.3	cPH[1]	99.8	h
Rom1p	Ygr070	1155	682-822	+	PH	cPH	99.1	cPH[1/2/3]	99.6	h,o
Rom2p	Ylr371w	1356	875-1020	+	PH	cPH	99.2	cPH[1/2/3]	99.5	h,o
Rpn13p	Ylr421c	156	20-132	Rpn13	Rpn13	–	100.	Rpn13	100.0	c
Rtt106p-1	Ynl206c	455	70-197	Rtt106	–	–	99.8	SSrecog	100.0	c
Rtt106p-2	Ynl206c	455	215-305	Rtt106	Rtt106	–	100.	Rtt106C	100.0	c
Sec3p	Yer008c	1136	107-223	–	PH	–	100.	cPH[sec3]	100.0	c
Sip3p-1	Ynl257c	1229	308-430	cPH	cPH	cPH	99.3	cPH[1/2/3]	99.9	h
Skg3p-1	Ylr187w	1026	89-230	cPH	cPH	cPH	97.3	cPH[1/2/3]	99.8	h
Skg3p-2	Ylr187w	1026	253-385	cPH	cPH	–	94.6	cPH[1/3]	97.4	h
Skm1p	Yol113w	655	03-122	cPH	cPH	cPH	99.3	cPH[1/2/3]	99.9	h
Slm1p	Yil105c	686	467-583	cPH	cPH	cPH	98.2	cPH[1/2/3]	99.9	h

continued overleaf

A (cont'd) protein name	systematic gene name	protein length	residues	method 1 InterPro-Scan	2 SMART	3 SUP-FAM	4 PDB-to-yeast p[SS]	4 PDB-to-yeast family	5 yeast-to-PDB	note
Slm2p	Ynl047c	656	445-555	cPH	cPH	cPH	98.3	cPH[1/2/3]	99.9	h
Spo14p	Ykr031c	1683	487-668	cPH	cPH	cPH	97.4	cPH[1/2/3]	99.7	h
Spo71p-2 °	Ydr104c	1245	741-970	cPH	5	–	93.4	cPH[1/2/3]	97.1	h
Spo71p-3 °	Ydr104c	1245	1026-1240	cPH	cPH	cPH	97.4	cPH[1/2/3]	97.0	h
Spt16p-1	Ygl207w	1035	550-660	+	+	–	100.0	Spt16D	100.0	c
Spt16p-2	Ygl207w	1035	665-820	–	–	–	100.0	Spt16M	100.0	c
Spt16p-3	Ygl207w	1035	835-940	Rtt106	Rtt106	–	**75.7**	Rtt106C	100.0	c
Ste5p	Ydr103w	917	400-510	–	–	–	86.5	cPH[2]	98.0	h
Syt1p	Ypr095c	1226	855-1075	cPH	cPH	cPH	**60.1**	cPH[1/2/3]	99.7	h
Tfb1p	Ydr311w	642	01-111	TFIIH	TFIIH	TFIIH	99.9	Tfb1	100.0	c
Tus1p	Ylr425w	1307	710-888	cPH	cPH	cPH	99.2	cPH[1/2/3]	99.8	h
Vps36p	Ylr417w	566	15-160	+	gram	gram	99.9	glue	100.0	c
Yel1p	Ybl060w	687	395-551	–	cPH	–	95.8	cPH[1/3]	99.8	h
Yhr131cp	Yhr131c	850	130-306	+	cPH	cPH	92.7	cPH[split]	99.9	h
Ymr1p	Yjr110w	688	16-130	+	0.03	gram	99.7	gram	97.5	h
Ynl144cp	Ynl144c	740	135-306	+	+	cPH	95.1	cPH[split]	99.9	h
Yrb1p	Ydr002w	201	80-199	RBD	PH	RBD	99.9	RBD[2]	100.0	h
Yrb2p	Yil063c	327	205-327	RBD	PH	RBD	99.9	RBD[2]	100.0	h
Ysp1p-1	Yhr155w	1228	305-430	cPH	cPH	cPH	99.4	cPH[1/2/3]	99.9	h
Ysp2p	Ydr326c	1438	632-760	gram	gram	–	97.3	gram	97.2	h
Known positives identified				62	59	48	71		72	
False positives (non-PH domains identified)				3§	0	0	1§		1§	

B New PH-like proteins	systematic gene name	protein length	residues	PSI e-val	PSI #	SMART	FFAS	PHYRE	PDB-to-yeast p[SS]	PDB-to-yeast family	Yeast-to-PDB p[SS]	Yeast-to-PDB PDB hit	HHalign
Age1p	Ydr524p	483	1-130						95	split	42.0	thiored.	0.1*
Bud2p-1	Ykl092c	1105	28-144 ¶	-6	3		-8.7	97	60.5	split	97.6	1upq_A	99.8
Bud2p-2	Ykl092c	1105	203-315	-24	3		-14	90	63.2	cPH[3]	99.8	1wi1_A	89.9
Caf120p-1	Ynl278w	1060	240-364	-27	1	+2	-11	96	97.4	cPH[1]	97.5	1dro_A	94.0
Gyp7p	Ydl234c	747	1-155				-12	25	66.1	Vps36	95.7	2cay_A	96.1
Lam1p-2	Yhr155w	1228	596-700	-14	2		-13	98	96.6	gram	96.5	2cay_A	97.9
Pkh1p §	Ydr490c	767	542-673						<5		97.9	1w1g_A	86.2
Pkh2p	Yol100w	1082	860-967	-2 T	2		-16	97	23.3	cPH[1]	98.1	1w1g_A	98.4
Rbh1p	Yjl181c	612	440-546				-11	64	<5		91.9	1rrp_B	88.4
Rbh2p	Yjr030c	746	566-680				-10	93	32.7	FERM1	95.8	1rrp_B	93.4
Rec114p	Ymr133w	429	1-130					68	18.3	RBD[1]	57.4	2oqb_A	71.0
Sip3p-2	Ynl257c	1229	599-699	-7	2		-14	98	96.0	gram	97.4	2cay_A	98.3
Spo71p-1	Ydr104c	1246	492-613	-18	1	-30	-11.9	97	<5		94.9	2dtc_A	94.5
Tph3p-1	Yjl016w	562	62-178				-6.1	97	<5		96.6	2rov_A	95.7
Tph3p-2	Yjl016w	562	182-407	-6	1	-9	-11	94	74.2	cPH[1]	88.4	2d9w_A	88.2
Vid27p-1	Ynl212w	783	16-165						<5[77]	indirect	51.5	UmVid27p-2	96.8
Vid27p-2	Ynl212w	783	228-339	-2 T	4		-29	98	96.2	RBD[2]	99.9	3m1i_B	99.8
Vps13p	Yll040c	3144	3028-144				-10	95	74.5	bactPH	94.1	3hsa_A	95.0

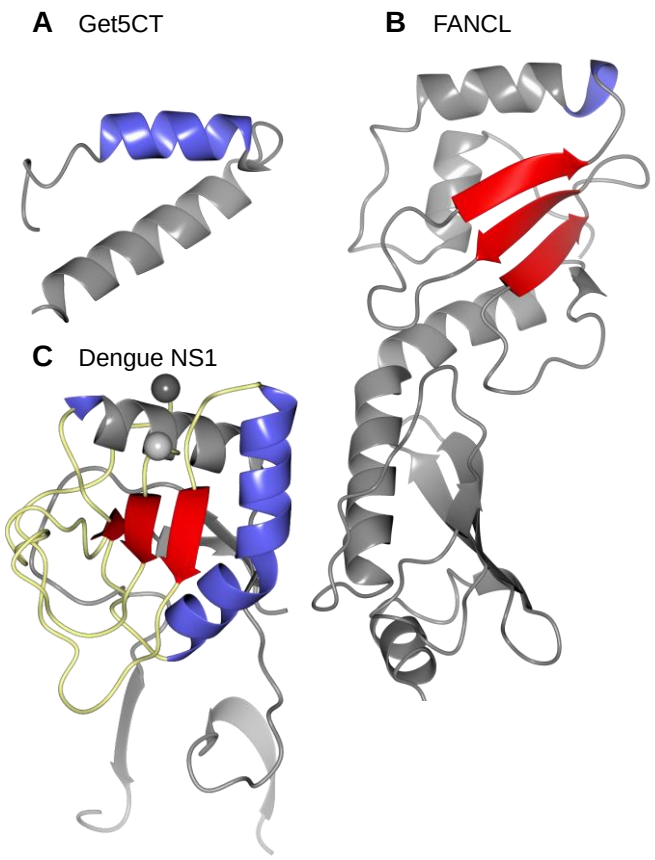
Supplemental Table 2. Description of PH-like domains in yeast

A. 73 known PH-like domains in yeast identified by 5 different methods: InterProScan (available through yeastgenome.org), Smart, SuperFamily (methods 1–3) and HHsearch (PDB-to-yeast and yeast-to-PDB =

methods 4 and 5). For methods 1-3, any family specified is indicated (cPH = classical PH), while "+" indicates a generic PH-like identification. § False positives in InterProScan were produced by Gene3D in Cbk1, Fpk1p and Kin82p. HHsearch identified Age1p as a false positive (see Supplemental Figure 4). For method 4, the prob[SS] and family (among the 39 we defined – see Figure 2) of the strongest hit to the yeast domain is indicated. Other notes: c = crystal structure solved, h = homologous to a solved structure, o = omitted by Yu *et. al*. (2004) [64]; ° numbering of PH-like domains within individual proteins takes account of the new domains we identify (see Figure 4).

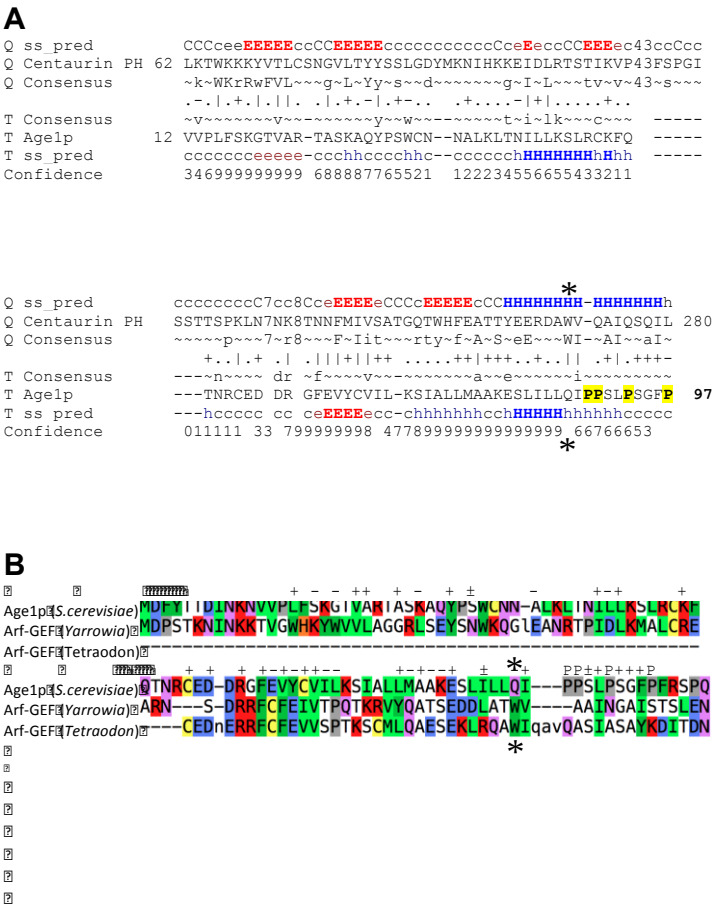
B. Newly identified PH-like domains in yeast. Details include discoverability by different tools: (1) PSI-BLAST, showing E-value ($\log_{10}$) and number of iterations (#) when a known PH-domain (definitions 1 or 2 including yeast proteins in part A above) is found in the hit list; ^{T} indicates only temporarily in hit list, lost before convergence; (2) SMART domain prediction server, showing E-values ($\log_{10}$) for any domains detected; (3) FFAS profile-profile tool, (score is more significant when more negative, threshold = –9) [47]; (4) PHYRE2 structural prediction tool (showing probability of PH-like fold, if top hit) [48]. HHsearch: method 5 = PDB-to-yeast (prob[SS]and query); method 6 = yeast-to-PDB (prob[SS] and target); method 7 = HHalign result with target in method 6. Prob[SS] values outlined in red were used to make identifications: 4 in PDB-to-yeast, 11 in yeast-to-PDB; one further identification was made by indirect searches (outlined in black). Other notes: ¶ Bud2p-1 is indicated by SMART as overlapping a false-positive C2 domain; § new true positive in Pkh1p already in SGD (identified by Gene3D); "T" indicates that for Pkh2p and Vid27p-2 the PSI-BLAST hit was only temporary, and was missing at convergence. Grey shading = non-significant hit in Rec114p found using ssw=30%; yellow = false positive for Age1p-N, where the top hit in yeast-to-PDB searches was a thioredoxin, and pairwise alignment of Age1p-N and the PH domain from centaurin (human ARFGEF) had prob[SH]=0.1%.

Fidler *et al.* Supplemental Figure 1.



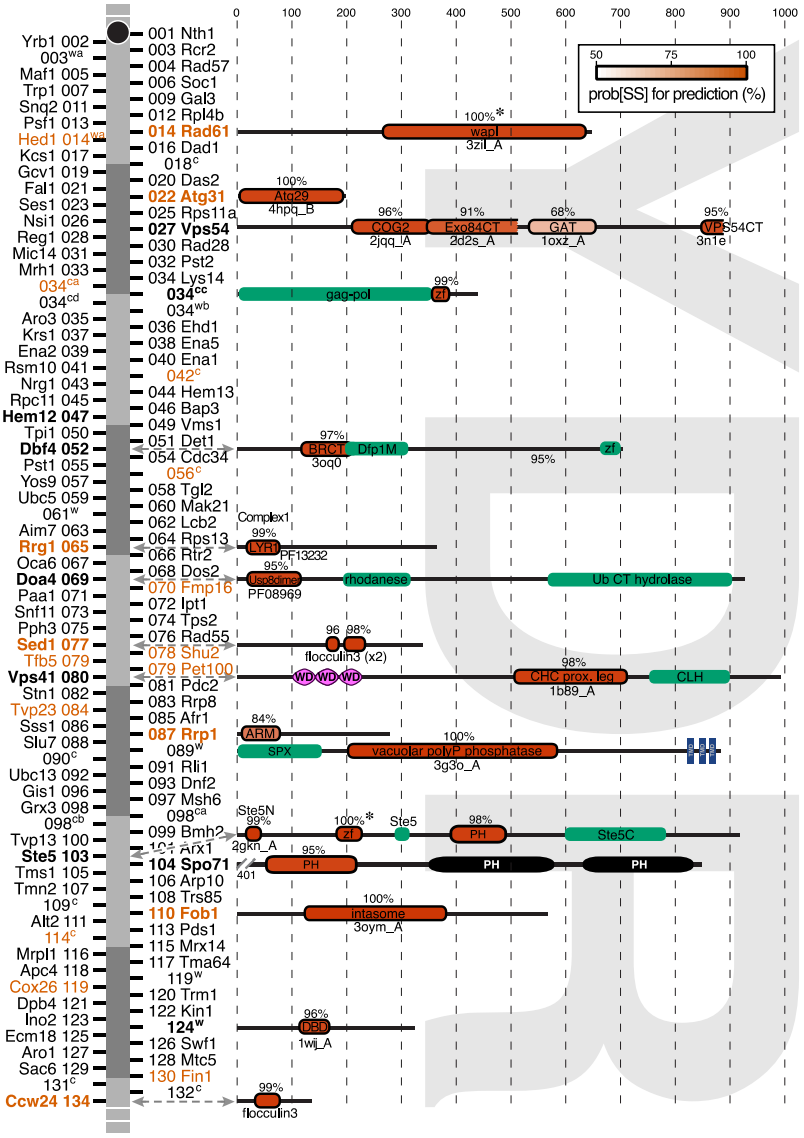
Supplemental Figure 1: Structures of strongest hits that are not PH-like domains
Three of the strongest false hits to non-PH-like proteins among PDB-to-PDB HHsearches with 39 PH-like domains (indicated as black squares in Figure 3A): **A.** C-terminus of yeast Get5p (3vej_A) aligned at 14 of its 41 residues to the helix of 4chj_A, prob[SS]=77%; **B.** mid-section of human FANCL (3zqs_A) aligned at 40 of its 186 residues to strands 3–5 of 2kig_A, prob[SS]=83%. **C.** $\alpha\beta$ subdomain of NS1 from Dengue virus type 2 (within a multi-domain structure of 350 aa, not shown) aligned at 90 of its 114 residues to all 7 sheets in 4gou_A, prob[SS]=76%. Aligned regions shown in colors (blue = helix, red = sheet, yellow = loop). While some non-PH-like hits are very short and can be identified as non-PH-like by that criterion (A), others cover multiple structural elements and cannot be excluded on the basis of shortness (B & C).

Fidler *et al.*, Supplemental Figure 2



Supplemental Figure 2: Dissection of a false positive in Age1p
A. Alignment showing a strong hit obtained in HHsearch between the PH-like domain of human ArfGEF Centaurin and the N-terminus of Age1p. For interpretation of the alignment, see Figure 5B legend. Three unstructured loops in centaurin of 43, 7 and 8 aa are omitted. The single-most conserved residue in PH-like domains is the tryptophan in the helix (asterisk), which is reported as making a very good hit (|) with a glutamine in Age1p. **B.** All sequence from the first 102 columns of the Age1p MSA in HHsuite. This part of the MSA includes only 3 of 164 sequences: Age1p N-terminus (1-102/482aa), *Y. lipolytica* ArfGEF (GI:50550125; 735-833/1099aa), and *T. nigroviridis* ArfGEF (GI:47212317; 425-474/991aa). Coloring by HHsuite AlignmentViewer. All residues where the HHsearch alignment in part A. reported a good or very good hit (+ or |) are scored for actual conservation between Age1p and the other sequence(s): +, ±, – for strength of hit. Residues 81-97 of Age1p predicted to align with alpha helix of centaurin in A. contains multiple prolines in its second half (highlighted in both A and B). The N-terminus of Age1 has homologs only in species very closely related to *S. cerevisiae*, so this part of the MSA should only have one sequence. However, the downstream GEF (Age1p residues 168-307) has 163 homologs, many of which have a domain structure BAR-PH-GEF. Two of these align to part of Age1p 1-130 (A). These give a PH-like appearance to the MSA at its N-terminus that is not present in the original seed (B).

Fidler *et al.* Supplemental Figure 3.



Supplemental Figure 3: New domains found in 132 consecutive yeast genes
132 consecutive verified open-reading frames on the long arm of chromosome IV ("YDR") starting at the centromere (YDR001C-134C) were analyzed by HHsearch. Names in bold indicate proteins (x16) where new domains were documented (x21). New domains are outlined in black and shaded by prob[SS] on a white–red scale; scores and main hit in PDB or Pfam are also given. All new hits were confirmed by pairwise alignments of domains alone. Asterisks indicate that the domain was detected by SMART even though absent from InterProScan data displayed at yeastgenome.org). Known domains in these proteins are shaded as in Figure 4 or in green. Naming: where a protein only has a systematic name, just the suffix is given (*i.e.* "XYZ $^{w/c}$" from the full name YDRXYZw/c). Red indicates the proteins (19 = 14%) without prior structural/functional predictions (discounting assignment to a fungal protein family and prediction of transmembrane domains). Seven of these (37%) had newly predicted domains. By contrast, nine of 113 (8%) previously characterised proteins had newly identified domains.

Movies 1-3: PH-like domains share structure
Rotating models of structures in Figure 1.